REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested. Claims 14-70 are in this case. Claims 14-70 have been rejected. Claims 14-63, 68 and 69 have now been canceled without prejudice. Claims 64, 66 and 70 have now been amended. New claims 71-79 have now been added. Claims 64-67 and 70-79 appear in the application.

Rejections over 35 USC 112, First paragraph

The Examiner has rejected claims 14-70 over 35 USC 112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s) had possession of the claimed invention. The rejections of the Examiner are respectfully traversed.

During the telephonic Interview with the Examiner held on February 3, 2004, the Examiner noted that these rejections stemmed from one of two sources. The first source is the inclusion of limitations which were not completely taught in the present Application (claims 14-63). While continuing to traverse the rejections of the Examiner in this regard, Applicant has chosen to cancel claims 14-63, 68 and 69 without prejudice in order to expedite the prosecution. Applicant believes that this response overcomes the Examiner's rejections in this regard.

The second source of the rejections is the inclusion of language concerning homology of less than 90% to SEQ ID NO: 10. Again, while continuing to traverse the rejections of the Examiner in this regard, Applicant has chosen to amend claims 64, 66 and 70, and to add new claims 71-79. These claims recite a limitation of homology of at

least 90% (claims 64-67, 70, 73, 75, 76 and 78) or at least 95% (claims 71, 72, 74, 75, 77 and 79). During the Interview, the Examiner indicated a willingness to consider limitations of homology of at least 90%, with particular favor to limitations of homology of at least 95%.

Applicant feels that both sets of limitations are allowable as follows. As described in the attached Appendix 1 (which is a Declaration of Iris Pecker that was submitted in another application by the same inventors, U.S. Serial Number 09/988,113), heparanase from mouse has a homology of about 80% to human heparanase, while heparanase from chick only has a homology of less than 70% to human heparanase. Yet these heparanase proteins are still clearly, recognizably heparanase, they retain heparanase functionality and they have a similar level of activity, in comparison to human heparanase. Thus, clearly heparanase proteins featuring a sequence of at least 90% homology to human heparanase are well within the level of homology that could be expected for heparanase, and that would result in a protein that has heparanase activity.

For purposes of further clarification, Applicant has submitted alignment data in the attached Appendix 1, showing the homology (and differences) between human, rat, mouse and chicken heparanase sequences. Some important shared features such as the heparan sulfate binding site are marked. This information further supports Applicant's statements with regard to the ability of one of ordinary skill in the art to readily recognize a heparanase protein as such. Furthermore, Applicant again notes that such homology can even be detected in a heparanase protein that has sequence homology of less than 70%; while for the present invention claims recite "at least 90%" homology. Thus, Applicant has limited the recited homology even further than that which could be predicted for

heparanase proteins, based upon the large amount of information that is available about other members of the heparanase family.

Support for these recitations can be found throughout the specification, particularly on page 59 thereof.

Rejections over 35 USC 102

The Examiner has rejected claims 64-67, 69 and 70 under 35 USC 102 as being anticipated by US Patent No. 5,362,641 to Fuks et al. (hereinafter "Fuks"). The rejections of the Examiner are respectfully traversed.

Fuks et al. describes the purification of a protein which, as described below, results in the production of a mixture of proteins, of which PAI1 is a significant component, even after all of the described purification procedures of Fuks et al. have been performed. In fact, later evidence has shown that the antibody raised by Fuks et al. against heparanase is actually an anti-PAI1 antibody.

Applicant noted these facts in Applicant's previous response. To further explain the important differences between the present claims and the teachings of Fuks, and to expedite the prosecution (while continuing to traverse the rejections of the Examiner), Applicant has chosen to cancel claim 69 without prejudice, and to amend claims 64, 66 and 70, while adding new claims 71-79.

In the Interview with the Examiner, the Examiner stated that Fuks taught a purification process which resulted in a mixture of proteins that presumably included heparanase. The Examiner agreed that (based upon later evidence, including a Declaration by Prof. Israel Vlodavsky, one of the inventors of the Fuks reference) the teachings of

Fuks would not result in purified heparanase. However, it is also obvious that Fuks does not teach any sequence of heparanase, or indeed any sequence of any protein.

In order for a rejection to be made under 35 USC 102, all of the limitations of the claim must be explicitly taught by the reference. Fuks does not teach the limitations of the present claims because Fuks does not teach or suggest particular sequences of any protein. Fuks et al. also does not teach or suggest homologies to a particular protein or amino acid sequence.

In short, Fuks et al. fails to teach or suggest any of the important limitations of the present claims. Furthermore, Fuks et al. also fails to render any of these claims obvious, because as previously described in the previously filed Response and Declaration, actually determining the sequence of heparanase (protein and polynucleotide) *for the first time* proved to be a non-trivial task. Applicant wishes to stress *for the first time* because once the initial sequence was correctly determined, locating other such sequences became a much simpler task. However, short of such an initial sequence determination, the teachings of Fuks et al. would not be sufficient to allow one of ordinary skill in the art to elucidate the sequence of heparanase without undue experimentation. The Examiner has failed to reject any of Applicant's previous arguments in this regard, so Applicant feels that the rejections of the present claims over Fuks should be removed. As Applicant noted previously these arguments are further supported by the decision of the court in *In re Duel* (34 USPQ2d at 1215), as follows:

> until the claimed molecules were actually isolated and purified, it would have been highly unlikely for one of ordinary skill in the art to contemplate what was ultimately obtained. What cannot be contemplated or conceived cannot be obvious.

As Applicant noted above, once *one* *__complete__* *sequence* was *correctly identified and isolated*, obtaining further such sequences would be simple. Fuks neither teaches nor suggests a single such sequence. The mere existence of a general method for isolating DNA molecules and determining their sequence was held by the court to be insufficient for a finding of obviousness.

In order to further support these points, Applicant has attached Declarations, identified as Appendix 2 and Appendix 3, submitted in a corresponding European patent application, EP 99 92 1513.0-2402 by two of the present inventors, one of whom (as previously mentioned) was also a co-inventor for the Fuks reference.

The argument that Fuks teaches a process which results in a mixture of proteins, and that somehow this argument anticipates or renders obvious the present claims, therefore clearly fails for the following reasons. First, as noted above, Fuks not only failed to obtain the sequence of heparanase, Fuks even failed to obtain pure heparanase. Indeed, in the mixture of proteins that Fuks obtained, heparanase is only a minor contaminant. This was later proved by Prof. Vlodavsky. This discrepancy can be explained by the fact that heparanase is by its nature a highly active enzyme; a trace contamination of heparanase protein in a mixture of other proteins can lead to the illusion of obtaining a highly pure preparation of heparanase.

The illusion is further sustained because of the methods used to monitor the taught purification procedure of Fuks. These methods relied upon following the activity of heparanase in column fractions, which can lead to the above drawbacks, and also upon following heparanase 'purification' through protein gels. The latter method is susceptible to a problem that Fuks never even suspected, which is that other proteins present in the

mixture (including PAI-1) have very similar properties on protein gels. These proteins tend to run at about the same apparent molecular weight as heparanase (as shown in Figure 5C, II of Fuks), leading to the appearance of a single broad band on the gel. One of ordinary skill in the art would therefore have been further lead astray by the apparent purity of the protein preparation as taught by Fuks.

In fact, the teachings of Fuks would cause one of ordinary skill in the art to fail to determine heparanase according to the present invention, as a large number of inventive acts are required to determine the sequence of heparanase.

The first such inventive act would be to realize that the teachings of Fuks are wrong. One of ordinary skill in the art would have no motivation to believe that the teachings of Fuks are wrong, as in fact these teachings give the highly misleading *appearance* of providing a successful procedure for isolating heparanase. These teachings are deceptive, as discussed above the taught process results in a heparanase preparation having a higher degree of specific activity than the starting preparation, and when run on a gel, the preparation provides the illusion of a single protein being present because a single band is present. Normally, one of ordinary skill in the art would rely upon these indicators as showing that the taught process does actually provide pure heparanase. Such a reliance would be wrong in this case, not because such indicators are never reliable, but rather because *in this specific case of heparanase*, the indicators are false. Heparanase is a very active enzyme, so the 'high' degree of specific activity does not actually show a pure preparation of heparanase, but one of ordinary skill in the art could never have predicted such an exception to the general rule. The presence of a mixture of proteins in the 'heparanase' band of the gel could also not have been predicted by one of ordinary

skill in the art, as the gel gives every *appearance* of showing a highly pure preparation. Only now, after the many inventive acts of the inventors, has such an appearance been shown to be false.

If the person skilled in the art nevertheless chose to perform the taught purification process of Fuks through the final suggested (but not performed) HPLC purification step, such a person would be confronted with a mixture of proteins, as demonstrated by the attached Appendix B, provided by the inventors of the present application, who did actually perform this step. The mixture would show heparanase activity, as in fact a portion of this mixture would include heparanase itself. However, it would also include other proteins, including PAI-1, a known contaminant of heparanase obtained through protein purification. Furthermore, the person skilled in the art would know that all previous purification processes that had been touted as producing "pure" heparanase actually resulted in the purification of many different proteins, but not the enzyme heparanase. Appendix C provides a listing of only a sample of some of the many different proteins which were thought to be "pure" heparanase.

The performance of tryptic digestion and microsequencing of the mixture of proteins would therefore result in multiple protein (amino acid) sequences being obtained. The inventors actually compared these sequences against the database of known proteins, and found that a number of such sequences did not match any known protein. These sequences are given in Appendix D, with some exemplary identified sequences from known proteins. Even at this stage, the person skilled in the art would presumably be questioning the presence of multiple unidentified sequences, particularly since only one, YGPDVGQPR, was found to match any EST.

Assuming that the person skilled in the art continued to attempt sequencing with the sequences, a further problem would arise. The EST sequences do not code for the entire protein. When a cDNA is cloned from these sequences with a "complete" protein sequence, however, a polypeptide is obtained which has 543 amino acids and a calculated molecular weight of 61,178 daltons. Since heparanase is known to be glycosylated, the molecular weight is expected to be even higher. However, Fuks teaches that the *expected* molecular weight of heparanase is 50 kD (col 15, lines 19-23 and 50-55). Therefore, the person skilled in the art would in fact believe that the wrong protein had been obtained, and would therefore be expected to cease further experiments with the "complete" protein sequence.

Even if the person skilled in the art chose to persevere, against the experimental evidence, which is also clearly an inventive act, further difficulties would arise if such a person attempted to actually produce heparanase *in vitro* from the cloned gene, for example in order to examine the activity of the gene product. Such a person might be expected to produce heparanase by transfecting yeast, for example. However, yeast cannot produce active heparanase, as demonstrated in Appendix E. Attempting to transfect a mammalian cell line would result in the problem of being unable to distinguish heparanase activity produced by the product of the transfected heparanase gene, and that of native heparanase, since the most commonly used mammalian cell lines have basal endogenous heparanase activity, as seen for example in the cell lines 293 and CHO (Chinese hamster ovary cells) . The person skilled in the art would thus assume that the heparanase "gene" actually did not code for heparanase, as no activity would be observed from transfection into yeast.

If the person skilled in the art continued to persevere, having committed a number of inventive acts in an attempt to obtain the heparanase sequence (as described above), such a person would now be required to perform a truly inventive act in order to obtain active heparanase from the suspected gene.

The person skilled in the art would now need to transfect insect cells with the heparanase gene sequence, using the Baculovirus expression system. This act is truly inventive because insect cells are not regularly used for mammalian proteins, and would certainly not be the cell line of choice after yeast failed to produce an active heparanase protein product. Appendix F demonstrates why insect cells are not usually a cell line of choice for mammalian proteins, and also some of the typical uses for insect cells with the Baculovirus expression system for gene transfection.

As described in the present Application, the use of insect cells with the Baculovirus expression system results in the production of active heparanase gene product. However, there is a further potential source of confusion for the person skilled in the art. The full length polypeptide for which the heparanase gene codes, which is of about 66 kDa, is actually a prepro-heparanase form of the protein, which actually has no heparanase activity.

A residual level of such activity can be detected due to the effect of non specific protease activity, which results in the activation of a minor fraction of the recombinant enzyme. The detection of heparanase activity was found to require several micrograms of recombinant enzyme, a quantity that is much higher than expected from a catalytic enzyme. This finding raised doubts about the functional identification of the cloned cDNA, and would clearly cause any person of skill in the art to doubt whether the correct gene had

been identified and cloned. Thus, if a person of skill in the art had actually performed the necessary experiments, such a person would have assumed that the true heparanase gene had not been identified.

Should the person of skill in the art suspect that the coded heparanase that had been identified is a pre or pro form of the protein, because the actual heparanase protein is about 51 kDa in size, and should that person then attempt to obtain an actual heparanase protein through protein cleavage, the resultant protein would actually be inactive. This apparently contradictory result would be obtained because two portions of the pro-heparanase protein must rearrange themselves to form the lower molecular weight heparanase, as described in Appendix G. The pro-heparanase protein can be divided into three sections: an 8 kDa section, a 6 kDa section and the main 50 kDa section. In order to produce heparanase, the 6 kDa section is removed and the 8 kDa section joins to the 50 kDa section. Thus, the person skilled in the art could not actually produce the 50 kDa protein through mere cleavage of the actual gene product, and would need to display a clearly inventive understanding of heparanase and of the gene itself in order to obtain the invention claimed in the present Application.

In any case, it should be noted that suspecting that the identified heparanase was a pre-pro form of the enzyme is in itself inventive, because the person of skill in the art would have no basis for such a suspicion. Beyond this suspicion, however, the person of skill in the art would need to understand how to ameliorate the problem. The present inventors were able to use insect cells with the Baculovirus system to overcome the twin problems of existing levels of heparanase activity in mammalian cells, and lack of

activity after transfection of yeast cells. However, this solution is by no means obvious, for the reasons given above.

Applicant would like to further point out to the Examiner that the present Application provides a full and complete description of all processes required to *reproduce* the present invention, since mere *reproduction* does not involve re-discovering the invention. As described above, the present invention actually overcomes a large number of problems, each of which represents an inventive act. Furthermore, within each inventive act, there are actually two or more problems, since the inventor would need to actually recognize the true nature of each problem. Even though the solution to each such problem is in itself difficult, the true difficulty is multiplied many times by the obscured nature of each problem. For example, at the very beginning of the inventive process described above, the cloned cDNA would be expected to produce a polypeptide with a calculated molecular weight of 61,178 daltons, although Fuks teaches that the *expected* molecular weight of heparanase is 50 kDa. The true nature of this problem is therefore obscure. Does the gene product have a different molecular weight because it codes for the wrong protein? Is the coded protein a pre or pro-heparanase? Or is some other factor responsible for this discrepancy? Indeed, does the gene product code for a protein that is even related to heparanase?

Applicant would also like to emphasize the impact of each prior stage on the expected interpretation of the next stage by the person skilled in the art. Continuing with the example of the previous paragraph, the apparently contradictory protein molecular weights would have been evaluated by the person skilled in the art in view of the microsequencing results. These results would have indicated that a mixture of proteins

was present, contrary to the teachings of Fuks which emphasize a very high degree of purity. Thus, the person skilled in the art would have understood the contradictory molecular weight as indicating that the wrong protein had been obtained.

Thus, Applicant feels that this overcomes the rejections of the Examiner in this regard.

For the reasons given above, Applicant feels that claims 64-67 and 70-79 are in condition for allowance. A prompt Notice of Allowance is respectfully requested.

Respectfully submitted,

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Date: April 19, 2004

Appendix B

The proteins identified by the inventors following the performance of Mono-S HPLC purification were found to be as follows: PAI-I, Nexin-I, Vimentin, Grp94/endoplasmin, FLT receptor, Tryptase.

Appendix C

The following is a list of only a sample of some of the many different proteins which were once erroneously thought to be "pure" heparanase:

1. PAI-I – antibodies generated against purified heparanase by Fuks & Vlodavsky (inventors of D3) were found to detect PAI-I rather than heparanase (Vlodavsky, personal)

2. CTAPIII -Hoogewerf et al. J Biol Chem 1995 Feb 17;270(7):3268-77

3. GRP94/endoplasmin (De Vouge et al. Int J Cancer 1994 Jan 15;56(2):286-94)

Appendix D

Peptides identified as *nexin*-1: (Vlodavsky, personal)

TFVAADGK
SENLHVSHILQK
SYQVPMLAQLSVFR
XGSTSAPNDLXYNFIE(?)XPY
LVLVNAVYFK
HNPTGAVLFM?XQI

Unindentified peptides: (Vlodavsky, personal)

XYGPDVGQPR
QVFFEAG?NYH?LVDENE
GLSPAYLR
XATDED(Y/L)(T/L)N(P/A)DV
VAASIYT
S?VQLF?(S/G)N(T/K)
SFLK
LLR

(not listed: sequences for peptides of the other identified proteins)

Appendix E

Expression of heparanase in E.coli, which is the easiest expression system and which would be the obvious choice for a person skilled in the art, results in unfolded or misfolded polypeptide which appears in inclusion bodies and which is obviously inactive. Yeast cannot produce active heparanase because no processing of pro-heparanase occurs in any microbial system, including that of yeast. Expression of full length heparanase cDNA in yeast results in secretion of a latent preproheparanase, which has no measurable activity (see for example US Patent No. 6348344: Genetically modified cells and methods for expressing recombinant heparanase and methods of purifying same).

Appendix F

Insect cells are not a typical choice for protein expression, since post-translational processing for mammalian proteins is typically not performed properly in insect cells. For the present invention, heparanase activity was detected due to a minor proteolytic activity of an endogenous insect cell non-specific protease. This resulted in a small fraction of partially processed active heparanase which could be detected due to the use of the most sensitive heparanase ECM assay. However, as described above, such a low level of activity would not be expected from a recombinant protein.

Appendix G

It should be noted that the exact, complete structure of heparanase is not currently known. It has been shown that mature active heparanase contains two subunits 45kDa and 8 kDa. The interaction is non-covalent and does not involve S-S bridges. There is no information regarding the location or chronology of processing events, although it has been shown that these two subunits are separated by another peptide in the pre-pro form of heparanase, in which the two subunits and the third peptide together form a single long peptide.